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Institute Report No. 339

Primary Dermal Irritation Potential of Triethyleneglycol Dinitrate (TEGDN) in Rabbits

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Primary Dermal Irritation Potential of Triethyleneglycol Dinitrate (TEGDN) in Rabbits (Toxicology Series 140)--Brown and Korte

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Edwin S. Beatrice 11 Jan 89

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The primary dermal irritation potential of triethyleneglycol dinitrate (TEGDN) was determined in male and female New Zealand White rabbits by using a modified Draize method. Very slight erythema was observed in 8 of 8 rabbits and very slight to slight edema in 5 rabbits by 1/2 to 1 hour after dosing. All rabbits had returned to normal by 72 hours after dosing. No other recognizable skin reaction was detected at any time during the 14-day observation period. The test compound was a mild irritant under conditions of this study.

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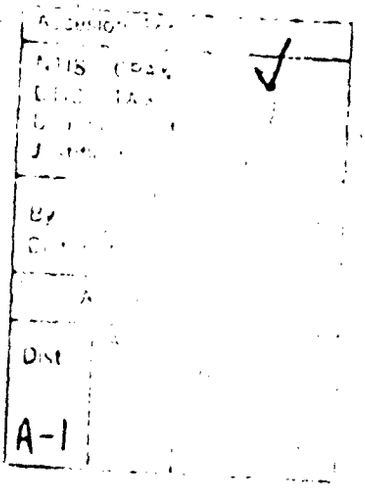
ABSTRACT

The primary dermal irritation potential of triethyleneglycol dinitrate (TEGDN) was determined in male and female New Zealand White rabbits by using a modified Draize method. Very slight erythema was observed in 8 of 8 rabbits and very slight to slight edema in 5 rabbits by 1/2 to 1 hour after dosing. All rabbits had returned to normal by 72 hours after dosing. No other recognizable skin reaction was detected at any time during the 14-day observation period. The test compound was a mild irritant under conditions of this study.

KEY WORDS: Primary Dermal Irritation, Triethyleneglycol Dinitrate, TEGDN, Mammalian Toxicology, Rabbit



PREFACE

TYPE REPORT: Primary Dermal Irritation GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, Maryland 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/AFC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 84045

STUDY DIRECTOR: MAJ Don W. Korte, Jr., PhD, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: LTC Larry D. Brown, DVM, VC
Diplomate, American College of Veterinary
Preventive Medicine, American Board of Toxicology

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Triethyleneglycol Dinitrate

INCLUSIVE STUDY DATES: 15 November - 18 December 1984

OBJECTIVE: The objective of this study was to determine the primary dermal irritation potential of Triethyleneglycol Dinitrate in male and female New Zealand White rabbits.

ACKNOWLEDGMENTS

Yvonne C. Johnson, BS, assisted in the research; SP4 James J. Fisher, SP4 Scott L. Schwebe, and Charlotte Speckman provided care for the animals; and Colleen S. Kamiyama, Brenda V. Goce, and Dianna Johnson provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84045 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

3 FEB89

DON W. KORTE JR., PHD / DATE
MAJ, MS
Study Director

11 Jan 89

LARRY D. BROWN, DVM / DATE
LTC VC
Principal Investigator

Dec 88

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA 3 January 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance Statement

1. This is to certify that the protocol for GLP Study 84045 was reviewed on 15 October 1984.

2. The institute report entitled "Primary Dermal Irritation Potential of Triethyleneglycol Dinitrate (TEGDN) in Rabbits," Toxicology Series 140, was audited on 10 August 1987.

Carolyn M Lewis

CAROLYN M. LEWIS, MS
Diplomate, American Board of Toxicology
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Primary Dermal Irritation Potential of Triethyleneglycol Dinitrate in Male and Female Rabbits–Brown and Korte

INTRODUCTION

The Department of Defense is considering the use of either diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL has tasked the Division of Toxicology, Letterman Army Institute of Research (LAIR), to conduct an initial health effects evaluation of the proposed replacement nitrate esters. This initial evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP, includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the primary dermal irritation potential of TEGDN in male and female New Zealand White rabbits.

MATERIALS

Test Substance

Chemical Name: Triethyleneglycol Dinitrate (TEGDN)

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code Number: TA44

Chemical Structure:

$O_2N\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}NO_2$

Molecular Formula: $C_6H_{12}N_2O_8$

Other test substance information is presented in Appendix A.

Animal Data

Four male and four female New Zealand White rabbits (Elkhorn Rabbitry, 5265 Starr way, Watsonville, CA), identified individually with ear tattooes numbered 84F696 to 84F703 inclusive, were assigned to the study. The animal weights on dosing day (4 Dec 84) ranged from 2.9 to 3.5 kg. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel, screen-bottomed, battery-type cages with automatically flushing dump tanks. The diet consisted of 150 g per day of Certified Purina Chow® Diet 5322 (Ralston Purina Company, Checkerboard Square, St Louis, MO), water was provided by continuous drip from a central line. The animal room temperature was maintained at 16.6 to 26.1°C with a relative humidity range of 32 to 62 percent with short spikes up to 84 percent associated with room cleaning. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study animals were acclimated for 14 days to the study room following a 14-day quarantine by the Animal Resources Group. During this period they were observed daily for signs of illness. They were treated prophylactically for ear mites with a single dose of Canex® and mineral oil instilled in the ears.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-34 (3).

The backs of 8 rabbits were close-clipped 24 hours before the actual dosing. The clipped area was divided into 4 quadrants designated I-IV (4, 5). Sites I and IV were sham patches. Sites II and III were test compound sites. Since TEGDN is a liquid, a standard dose of 0.5 ml of the test compound was placed on 1-inch (2.5 cm) square gauze patches that were taped to the appropriate sites. Blenderm® (Medical Products Division of 3M, Saint Paul, MN), a semi- impervious, hypoallergenic surgical tape, was used to hold the patches in place. Vet Wrap® (Animal Care Products Division of 3M, Saint Paul, MN) was then wrapped securely around the animal. The test compound was left in contact with the skin for 4 hours. At the end of the exposure period the wrapping and patches were removed, and the areas were scored 1/2 - 1 hour later.

Observations

The grading and scoring for dermal reactions were performed according to Table 1. Scoring and grading were performed at approximately 1/2 - 1, 24, 48, and 72 hours after removal of the patch. Observations for clinical signs were made daily from 4 to 18 December 1984. After 14 days the animals were submitted for necropsy and sections were taken from the application site for microscopic evaluation.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

Two animals were dosed with positive control compounds in conjunction with the eight animals in the study. This was part of a pilot study to assess different positive controls for incorporation in future studies. Findings from the necropsy of these two animals are included in the pathology report.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound were retained in the LAIR Archives.

TABLE 1 (4)
EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation		
No erythema	0	
Very slight erythema (barely perceptible)	1	
Well-defined erythema	2	
Moderate-to-severe erythema	3	
Severe erythema (beet-redness to slight eschar formation [injurious in depth])	4	
Possible total erythema score		**4**
Edema Formation		
No edema	0	
Very slight edema (barely perceptible)	1	
Slight edema (edges of area well-defined by definite raising)	2	
Moderate edema (edges raised approximately 1 mm)	3	
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4	
Possible total edema score		**4**
Possible total score for primary irritation		**8**

RESULTS

Animals were scored for erythema and edema at each patch site. All eight rabbits exhibited very slight erythema (score 1) at a minimum of one test compound site during the study (Appendix D). Six rabbits were observed to have very slight erythema and five rabbits had very slight to slight edema at 1/2 to 1 hour after dosing. Seven rabbits exhibited very slight erythema 24 hours after dosing. All rabbits except one had returned to normal by 48 hours after dosing. This rabbit returned to normal by 72 hours. No other recognizable skin reaction was detected at any time during the 14-day observation period. The sham patch sites were normal throughout the study. Total scores (erythema plus edema) for the dermal irritation potential in each rabbit were tabulated (Appendix D). Fourteen days after topical application there were no gross or microscopic skin lesions that could be attributed to exposure to the test material (Appendix E).

DISCUSSION

The modified Draize dermal irritation test as performed for this study has proven reliable for detecting non-irritating substances and severe irritants but considerably less reliable for detecting mild and moderate irritants (5). Consequently, many systems have been used to score and categorize the dermal irritation potential of a test compound. The system used by the Toxicity Testing Program at LAIR is an adaptation of one used at the U.S. Army Environmental Hygiene Agency (6). It develops a dermal irritation index based on the peak net mean score, which is the maximum net mean score calculated during the 72-hour observation period. Non-irritating compounds have peak net mean scores of 0.0 to 0.5. Mild irritants have peak net mean scores of 0.51 to 2.0. Moderate irritants have peak net mean scores of 2.1 to 5.0. Severe irritants have peak net mean scores of 5.1 to 8.0. TEGDN produced very slight erythema in 8 of 8 rabbits and very slight to slight edema in 5 of 8 rabbits. The peak net mean score for the test compound was 1.625; therefore, TEGDN was classified as a mild irritant.

Jones *et al* (7) studied the toxicity of propyleneglycol-1,2-dinitrate (PGDN), a liquid nitrate ester structurally similar to TEGDN and TMETN. Their studies showed that PGDN was absorbed percutaneously but produced no primary dermal irritation at either 24 or 72 hours after exposure to vapor. The results of this study suggest that TEGDN may have a greater potential to produce dermal irritation than PGDN. This finding is consistent with that reported by Brown *et al* (8). They reported that TEGDN produced dermal irritation in an acute dermal toxicity study. They also reported that TEGDN produced mild inflammation in an ocular irritation study. However, this inflammation, although scorable, was not sufficiently severe to be considered a "positive response"; therefore, TEGDN was classified as a nonirritant with respect to the eye.

CONCLUSION

The test compound TEGDN is a mild irritant under conditions of this assay.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.

2. Environmental Protection Agency. Office of Pesticide and Toxic Substances, Office of Toxic Substances (TS-792). Primary dermal irritation. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

3. Primary dermal irritation study. LAIR Standard Operating Procedure OP-STX-34, Presidio of San Francisco, CA: Letterman Army Institute of Research, 1 August 1984.

4. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 83:377-390.

5. McCreesh AH, Steinberg M. Skin irritation testing in animals. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology. 3rd ed. Washington, DC: Hemisphere Publishing Corp, 1987: 153-172.

6. U.S. Army Environmental Hygiene Agency. Topical hazard evaluation program. Procedural guide. Aberdeen Proving Ground, MD: U.S. Army Environmental Hygiene Agency, October 1985.

7. Jones RA, Strickland JA, Siegel J. Toxicity of propylene glycol 1,2-dinitrate in experimental animals. Toxicol Appl Pharmacol 1972; 22: 128-137.

8. Brown LD, Hiatt GFS, Morgan EW, Wheeler CR, Lewis CM, Johnson YC, Ryabik JRG, Okerberg CV, Makovec GT, Lollini LO, Mellick PW, Korte DW. Acute toxicity of TEGDN and TMETN liquid propellants. Laurel, MD: Chemical Propulsion Information Agency, 1985; CPIA Publication 436, pp. 313-320.

Appendix A: CHEMICAL DATA

Chemical Name: Ethanol, 2,2'-[1,2-ethanediylbis(oxy)] bis-, dinitrate

Alternate Chemical Names: Triethyleneglycol dinitrate, NOSET-A

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code Number: TA44

Chemical Structure:

$$O_2N\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}NO_2$$

Molecular Formula: $C_6H_{12}N_2O_8$

Molecular Weight: 240

Physical State: Yellow oil

Density: (g/cm^3): 1.32*

Manufacturer: Naval Ordnance Station
Indian Head, MD

Lot No.: 130-84

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* Holleman JW, Ross RH, Carroll JW. Problems definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846, p17.

Appendix A (cont.): CHEMICAL DATA

Analytical data: The compound chromatographed as a single peak (retention time 5.8 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% methanol; flow rate 0.9 ml/min, detection wavelength, 215 nm.† No impurities were detectable by NMR.† NMR (80 MHz, $CDCl_3$): 3.65 (S, 4H, $-CH_2-O-CH_2CH_2-O-CH_2-$), 3.72-3.84 (Complex multiplet, 4H, terminal methylene groups). IR (KBr): 2900, 1630, 1280, 1130, 1030, 910, 860 cm.§

Stability: The compound was received as a 10% solution in ethanol. Periodic analysis of this solution by HPLC has shown no evidence of decomposition to date (4 months).† NMR analysis demonstrated that the neat compound is stable for at least 1 month.†

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† Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p26-30, 42-43. Letterman Army Institute of Research, Presidio of San Francisco, CA.

† Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p63. Letterman Army Institute of Research, Presidio of San Francisco, CA.

§ Ibid. p64.

Appendix B: ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male and female

Age: Young adults

Animals in each group: 4 males and 4 females

Condition of animals at start of study: Normal

Body weight range at dosing: 2.9 to 3.5 kg

Identification procedures: Ear tag, tag numbers 84F696-84F700 inclusive.

Pretest conditioning:

1. Quarantine from 15 November - 28 November 1984
2. Animal were close-clipped and examined 24 hours before dosing.

Justification: Laboratory rabbits are a proven sensitive animal model for dermal irritation.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
15 Nov 84	Rabbits arrived at LAIR and were examined and caged.
16 Nov 84	Animals were tattooed, weighed, and placed under a 2-week quarantine.
15 - 28 Nov 84	Animals were checked daily by Animal Resources Group (ARG) personnel.
28 Nov 84	All rabbits were treated with Canex® and mineral oil in their ears to prevent ear mites. Rabbits were removed from quarantine after being certified healthy by ARG Staff Veterinarian. The animals were weighed.
28 Nov - 3 Dec 84	Animals were checked daily.
3 Dec 84	Animals were close-clipped and areas marked.
4 Dec 84	Animals were weighed. Test substance was applied for 4 hours. Patches were removed and sites scored within 60 minutes.
5 - 18 Dec 84	Animals were observed daily.
5 - 7 Dec 84	Areas were scored at 24, 48, and 72 hours after exposure.
16,23,29 Nov - 4,11,18 Dec 84	Animals were weighed.
18 Dec 84	Animals were weighed and sacrificed. At necropsy, skin tissues were collected for microscopic evaluation.

Appendix D: DERMAL IRRITATION DATA

ANIMAL NUMBER	OBSERVATION	QUADRANT* I	II	III	IV
84F696	30-60 min	0/0†	1/0	0/0	0/0
	24 hr	0/0	1/0	0/0	0/0
	48 hr#	0/0	0/0	0/0	0/0
84F697	30-60 min	0/0	0/0	0/0	0/0
	24 hr	0/0	1/0	0/0	0/0
	48 hr#	0/0	0/0	0/0	0/0
84F698	30-60 min	0/0	0/0	0/0	0/0
	24 hr	0/0	1/0	0/0	0/0
	48 hr	0/0	1/0	0/0	0/0
	72 hr#	0/0	0/0	0/0	0/0
84F699	30-60 min	0/0	0/2	1/0	0/0
	24 hr	0/0	1/0	1/0	0/0
	48 hr#	0/0	0/0	0/0	0/0
84F700	30-60 min	0/0	1/2	1/1	0/0
	24 hr#	0/0	0/0	0/0	0/0
84F701	30-60 min	0/0	1/0	1/1	0/0
	24 hr	0/0	1/0	1/0	0/0
	48 hr#	0/0	0/0	0/0	0/0
84F702	30-60 min	0/0	1/0	1/1	0/0
	24 hr	0/0	0/0	1/0	0/0
	48 hr#	0/0	0/0	0/0	0/0
84F703	30-60 min	0/0	0/1	1/0	0/0
	24 hr	0/0	1/0	1/0	0/0
	48 hr#	0/0	0/0	0/0	0/0

* Quadrant I, IV=sham; II, III=treated

† Scores are displayed as erythema/edema

Scores were 0/0 in all quadrants for remaining observations

Appendix D (cont.): DERMAL IRRITATION DATA

SUMMARY OF PRIMARY IRRITATION TEST DATA

Animal Number	30-60 min Test	30-60 min Sham	24 h Test	24 h Sham	48 h Test	48 h Sham	72 h Test	72 h Sham
84F696	1	0	1	0	0	0	0	0
84F697	0	0	1	0	0	0	0	0
84F698	0	0	1	0	1	0	0	0
84F699	3	0	1	0	0	0	0	0
84F700	3	0	0	0	0	0	0	0
84F701	2	0	1	0	0	0	0	0
84F702	2	0	1	0	0	0	0	0
84F703	2	0	1	0	0	0	0	0
Mean	1.625	0	0.875	0	0.125	0	0	0
Net Mean Score*	1.625†	0	0.875	0	0.125	0	0	0

*Test Mean - Sham Mean = Net Mean Score

†The peak net mean score is 1.625; therefore, TEGDN is a **MILD IRRITANT** (Table 1)

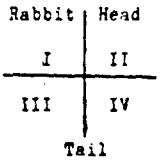
Appendix E: PATHOLOGY REPORT

Patnology Report
GLP Study 84045

Primary Dermal Irritation Test of triethyleneglycol dinitrate (TEGDN) in Rabbits

1. Purpose: To determine the primary dermal irritation potential of TEGDN in Rabbits (CAS No. 111-22-8).

2. Procedure: Five male and 5 female New Zealand white rabbits were used in this study. The skin of the back of each animal was clipped and divided into four quadrants:



The test substance was applied to quadrants II and III of each rabbit. No treatment other thar a dry patch was applied to quadrant I (shan control). Vehicle only (saline) was applied to quadrant IV. The test substance was kept in contact with the skin for 4 hours and reactions were scored according to the protocol. After a two-week observation period, the rabbits were killed by overdose of pentobarbital administered intravenously and a complete gross necropsy was performed. Strips of skin from each of the four quadrants were removed, fixed in 10% neutral buffered formalin, and processed for histologic examination according to OP-PSG 13, Histopathology-Tissue Processing, and stained with hematoxylin and eosin.

Three animals were used as controls for this study. Animal #84F695, Pathology Accession #LAIR 36385, was submitted for quality control necropsy on 16 November 1985, and there was no gross or microscopic evidence of intercurrent disease which would preclude its use for short-term dermal irritation studies. Rabbit #84F694, Pathology Accession #LAIR 36492, was used as a positive control. Two quadrants were treated topically with methyl ethyl ketone (MEK) and two quadrants treated with formalin. A fifth area was left untreated. Rabbit #84F709, Pathology Accession #LAIR 36497, was used as a second positive control. Two areas of skin were treated topically with benzoic acid and a third quardrant left untreated.

3. Gross Necropsy Findings:

Rabbit No.	Pathology Accession No.	Gross Necropsy Findings
84F694	36492 (positive control)	Quadrant I (MEK) slight edema area approximately 4 x 4 cm. Quadrant II (formalin) a 4 mm white raised area.

Appendix E (cont.): PATHOLOGY REPORT

GLP Study 84045

Rabbit No.	Pathology Accession No.	Gross Necropsy Findings
84F694 (Cont)	36492	Quadrant IV (MEK) slight erythema, slight edema, approximately 4 x 4 cm. Red crusty indented area approximately 1 cm in diameter.
		Cecum - contained worms.
84F703	36493	No lesions
84F702	36494	Cecum - contained pin worms.
		Ears - purulent otitis media, bilateral.
84F701	36495	Ears - purulent otitis media, bilateral.
84F700	36496	Ears - purulent otitis media, bilateral.
84F709	36497 (positive control)	No lesions.
84F699	36498	Cecum - contained pin worms. Ears - purulent otitis media.
84F697	36499	No lesions.
84F696	36500	No lesions.
84F698	36501	Cecum - contained pin worms.

4. Microscopic findings. Lesions are described in detail in attachment #1.

Pathology Accession No.	Rabbit No.	Quadrant
36492	84F694	1. Healing ulcer with extensive dermal fibrosis.
		2. Healing ulcer with extensive dermal fibrosis.
		3. Healing ulcer with extensive dermal fibrosis.

Appendix E (cont.): PATHOLOGY REPORT

GLP Study 84045

Pathology Accession No.	Rabbit No.	Quadrant	
36492 (Cont)	84F694	4.	Healing ulcer with extensive dermal fibrosis.
		5.	NLR
36943	84F703	1.	NLR
		2.	NLR
		3.	NLR
		4.	NLR
36494	84F702	1.	NLR
		2.	NLR
		3.	NLR
		4.	NLR
36495	84F701	1.	NLR
		2.	NLR
		3.	NLR
		4.	Dermatitis, subacute, focal, minimal, superficial dermis, skin.
36496	84F700	1.	NLR
		2.	NLR
		3.	Dermatitis, subacute, focal, minimal, superficial dermis, skin.
		4.	NLR.

Appendix E (cont.): PATHOLOGY REPORT

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Pathology Accession No.	Rabbit No.	Quadrant
36497	84F709 Quadrants 1 & 2 were the benzoic acid control sites. Quadrant 3 was untreated.	1. NLR 2. Dermatitis, subacute, focal, minimal, superficial dermis, skin. 3. NLR. Only 3 sections submitted.
36498	84F699	1. Dermatitis, subacute, focal, minimal, superficial dermis, skin. 2. NLR 3. NLR 4. NLR
36499	84F697	1. NLR 2. NLR 3. NLR 4. NLR
36500	84F696	1. NLR 2. NLR 3. NLR 4. NLR
36501	84F698	1. NLR 2. NLR 3. NLR 4. NLR

Appendix E (cont.): PATHOLOGY REPORT

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5. Comments:

There were no gross or microscopic lesions in the skin of rabbits in this study that could be attributed to exposure to the test material. One of 16 TEGDN-exposed sites in 8 rabbits did have very mild subacute focal dermatitis. However, 2 of 16 control sites also had this lesion. The lesion is very mild, very small, non-specific, and of a more recent duration than topical exposure 2 weeks previously would be expected to produce.

The two positive control substances, MEK and formalin, used on rabbit #84F694, both produced ulcerative skin lesions which were detected 14 days after they were applied. The lesions were of a similar type but differed in severity. Both of the lesions from sites treated with MEK were more severe and extensive than the formalin-induced lesions. Benzoic acid, however, did not cause any gross or microscopic change that could be detected 14 days after topical application.

Nematode parasites (pin worms) were observed in four rabbits at necropsy. These are common parasites of rabbits belonging to either Genus Dermatoxys or Passalurus, neither of which are considered pathogenic except in very heavy infestations. Their presence would not affect the results of a dermal irritation study.

Four rabbits on this study and the animal submitted for quality control necropsy had bilateral purulent otitis media. This condition is quite common in rabbits obtained from Elkhorn Rabbitry and other commercial sources. It is most likely due to infection by bacteria (Pasteurella multocida). This lesion is considered an indicental finding unrelated to the application of the test material. The lesion would not affect the results of this dermal irritation study.

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Appendix E (cont.): PATHOLOGY REPORT

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Attachment #1
Description of Histologic Lesions

1. Healing ulcer with extensive dermal fibrosis.

The lesion is characterized by severe necrotizing and inflammatory processes involving both epidermis and dermis and by the subsequent healing and regenerative processes in these areas. Depending upon the duration of the process, one or more of the following morphologic features may be present:

A. Epidermal changes. Acute lesions are characterized by necrosis or complete absence of all layers of the epidermis and the presence of numerous heterophils, many of which are degenerating fibrin and dessicated proteins which form a scab. At the edges of the lesion thin layers of epidermal cells are present beneath the scab. Peripheral to this area the epidermis may be quite thickened. The surface often contains flakes of keratin, some of which may be incorporated into the material forming the scab.

B. Dermal changes. Many of the adnexal structures beneath the ulcerated area are replaced by proliferating papillaries and fibrous connective tissue scar. Adnexa at the periphery may be encircled by the connective tissue proliferation and show signs of degeneration and atrophy.

2. Dermatitis, subacute, focal.

This lesion consists of tiny focal areas of necrosis in the capillary dermis with infiltration of heterophils and lymphocytes. Dermal collagen bundles in affected areas are fragmented and may be separated by clefts.

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